Bond Orientational Order, Molecular Motion and Free Energy of High Density DNA Mesophases

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Abstract

By equilibrating condensed DNA arrays against reservoirs of known osmotic stress and examining them with several structural probes, it has been possible to achieve a detailed thermodynamic and structural characterization of the change between two distinct regions on the liquid crystalline phase digram: a higher-density hexagonally packed region with long-range bond orientational order in the plane perpendicular to the average molecular direction; and a lower-density cholesteric region with fluid-like positional order. X-rays scattering on highly ordered DNA arrays at high density and with the helical axis oriented parallel to the incoming beam showed a six-fold azimuthal modulation of the first order diffraction peak that reflects the macroscopic bond-orientational order. Transition to the less-dense cholesteric phase through osmotically controlled swelling shows the loss of this bond orientational order that had been expected from the change in optical birefringence patterns and that is consistent with a rapid onset of molecular positional disorder. This change in motion was previously inferred from intermolecular force measurements and is now confirmed by ^{31}P NMR. Controlled reversible swelling and compaction under osmotic stress, spanning a range of densities between $\sim$ 120 mg/ml to $\sim$ 600 mg/ml, allows measurement of the free energy changes throughout each phase and at the phase transition, essential information for theories of liquid-crystalline states.

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Double helical DNA has emerged as a remarkably useful material for visualizing liquid-crystalline structures [1] and for measuring the packing energies associated with them. Robust DNA double helices, of almost any monodisperse length from a few base pairs to molecular weights $\approx 10^9$ can be obtained through modern molecular biology methods and can be condensed into highly ordered arrays easily probed by x-ray diffraction. The strong polyelectrolyte interactions between helices can be controlled effectively by type and concentration of the excess electrolytes. The form of the interhelical potential suggests that the lessons learned from concentrated DNA arrays will have broad applications to other seemingly unrelated physical systems such as their recently noticed similarity to magnetic vortex arrays in type II superconductors [2].

Given the extensive investigations of the physical properties and structure of condensed DNA phases, it is perhaps surprising that there has not yet been a comprehensive thermodynamic characterization of DNA mesophases under controlled solution conditions. Following Robinson's [3] seminal observation of a cholesteric-like phase of long DNA *in vitro* there have appeared several studies detailing the complexity of DNA phase behavior and its relevance for the conditions *in vivo* [1]. The sequence of mesophases for short-fragment DNA (146 bp $\sim$ 50 nm nucleosomal DNA) appears to be: isotropic solution $\longrightarrow$ cholesteric $\longrightarrow$ columnar hexagonal $\longrightarrow$ hexagonal [4]. With biologically more relevant long-fragment DNA ($\sim$ 100 nm to $\sim$ mm), the sequence of phases is less well delimited and characterized: isotropic solution $\longrightarrow$ ("precholesteric" $\longrightarrow$) cholesteric $\longrightarrow$ columnar hexagonal $\longrightarrow$ hexagonal crystalline [5]. These sequences were obtained on stoichiometric mixtures of DNA, salt, and water where there is often more than one phase present and where neither salt nor water chemical potentials are known.

A separate line of study of the condensed phases of DNA was initiated by Lerman [6] through the polymer and salt induced condensation (ψ DNA) and equilibrium sedimentation [7] of DNA solutions. The density of the condensed DNA was shown to depend continuously on the concentration of condensing polymer agent (usually polyethylene glycol, PEG) [8]. The use of *osmotic stress* [9] was built on the realization that the condensing polymer is essentially fully excluded from the DNA phase and that, at equilibrium, the activities of the exchanging water and salt are equal in the DNA and PEG phases [10]. Knowing the osmotic pressure (Π) contribution from the excluded polymer, measured by standard procedures as a function of its concentration, means the osmotic pressure of the DNA is also known while all other intensive variables such as pH and the chemical potentials of salt and other small solutes [9, 10, 11] are held fixed. Using x-ray scattering to measure the interaxial spacing, D_{int}, between double helices, this method was used successfully to elucidate a $\Pi - D_{int}$ dependence for DNA as a function of temperature, salt type and salt concentration [10, 11]. The range of osmotic pressures accessible through this method is substantially larger (especially at high stress) than by the equilibrium sedimentation approach [7].

Since DNA is equilibrated against a vast excess of a polymer and water solution of

known chemical potential, it is always in a single phase at thermodynamic equilibrium. This behavior should be contrasted with multiple phase equilibria that usually emerge from stoichiometric mixtures.

In this work we combine both the structural and thermodynamic approaches to the condensed DNA phases so that structural and dynamical parameters of DNA packing and ordering (interhelical separation, bond orientational order parameter, ^{31}P-NMR spectra) are all measured concurrently with the free energy and/or its derivatives. We report here the structural and dynamic changes that occur in the DNA concentration region from 120 to 600 mg/ml corresponding to interaxial separations of 25 to 50 Å. We show that at lower densities (or higher spacings) DNA packing is characterized by short range positional order, measured by x-ray diffraction, long range cholesteric order, revealed by optical birefringence, and high mobility of the DNA backbone, inferred from ^{31}P NMR spectrometry. At high densities (or small spacings) DNA packing is characterized by short range positional order and long range bond orientational order in the plane perpendicular to the average nematic director, revealed by the azimuthal profile of the first order x-ray diffraction peak and low mobility of the DNA backbone.

1 MATERIALS AND METHODS

Wet-spun oriented samples were prepared from calf-thymus DNA (Pharmacia) with a molecular weight of $\sim 1.6 \times 10^7$ by the standard method [12]. This spinning allows controlled production of sufficient amounts of highly oriented thin films by spooling DNA fibres which are continuously stretched during precipitation into an aqueous alcohol solution. Films of thickness of ~ 0.5 mm and surface area between 5 and 10 mm^2 were used in the experiments reported here.

Unoriented fibers of high MW ($\sim 1 \times 10^8$) DNA were prepared from whole adult chicken blood (Truslow Farms, Chestertown, MD) as described in McGhee *et al.* [13]. This DNA was further purified with three extractions against phenol/chloroform (50:50) and once with chloroform alone. Then DNA was ethanol precipitated in sodium acetate, pelleted by centrifugation, washed twice with 70% ethanol and dried. This DNA was used in all preparations involving unoriented fibers.

Oriented as well as unoriented DNA fibers were equilibrated with various solutions of PEG (20,000 MW) in 0.5 M NaCl, 10 mM Tris/ 1 mM EDTA, pH 7 in vast excess. Under these conditions, PEG (20,000 MW) is completely excluded from the DNA phase for concentrations greater than $\approx 7\%$(w/w). The equilibration time was usually from four days to a week. Measurements on both orientationally ordered (wet-spun) as well as "powder" samples show that there is essentially no difference in osmotic pressure vs. concentration (interhelical spacing) dependence between the two preparations. The two preparations differ only in the size of the oriented domains.

X-ray diffraction was performed at 20°C with an Enraf-Nonius Service Corp. (Bohemia, NY) fixed-anode FR 590 x-ray generator equipped with image plate detectors. Image plates were read and digitized by a Phosphor Imager (Molecular Dynamics, CA) and processed with NIH Image 1.55 program (W. Rasband, NIH, Bethesda, MD) modified by us. The position of the first order diffraction peaks ($r_{1.max}$) is obtained by radially averaging the scattering profile around the direct beam. Angular intensity profiles were taken at the position of the maximum of the first order diffraction peak and were then Fourier transformed to extract the bond orientational order parameter $\mathcal{C}_6$, *i.e.* the sixth order Fourier coefficient. If there were perfect alignment of the x-ray beam and the average director of the oriented DNA sample , the angular dependence of the six-fold symmetric scattering function could be Fourier analyzed in terms of [14]

$$\mathcal{S}(\theta, r_{1.max}) = I_0(r_{1.max}) \left[\tfrac{1}{2} + \sum_{n=1}^{\infty} \mathcal{C}_{6n} \cos 6n(\theta - \theta_0) \right] + I_{BG}, \qquad (1)$$

where I_{BG} is the background intensity. Because the orientation was only approximate there was usually a small $\mathcal{C}_2$ component present in the Fourier analyzed angular profiles. We have rescaled the value of $\mathcal{C}_6$ to correct for this .

DNA samples at various densities were sealed between microscope cover glass and were observed under a microscope (Olympus) equipped with crossed polarizers. The image was digitized and analyzed with NIH Image 1.55. The "fingerprint" cholesteric pattern [19] with long fragment DNA was never as regular as is typical of short fragment DNA. Rather long DNAs achieve oriented domains of much smaller size.

The ^{31}P NMR measurements were performed on a Bruker MSL-300 spectrometer (Billerica, MA) using a high power probe with a 5 mm solenoidal sample coil which was doubly tuned for ^{31}P (121.513 MHz) and protons (300.13 MHz). Gated broadband decoupled ^{31}P spectra were observed with a phase cycled Hahn echo sequence. A delay time between the 90 degree pulse and 180 degree pulse of 30 microseconds was chosen. Typically 20,000 to 80,000 scans with a recycle delay time of 1s were accumulated. Exponential linebroadening with a linewidth of 200 Hz was used.

First moments of the NMR spectra (M_1) were calculated in standard fashion according to

$$M_1 = \frac{\int_{-\infty}^{+\infty} f(\omega)\ \omega d\omega}{\int_{-\infty}^{+\infty} f(\omega)\ d\omega}, \tag{2}$$

where $f(\omega)$ is the spectral intensity at the frequency ω. The frequency of the center of the spectrum, determined as half height of the integral $\int_{-\infty}^{+\infty} f(\omega)\ d\omega$, was set to zero.

The measured dependence of the osmotic pressure of the DNA phase on DNA concentration allows one to evaluate the reversible work done at constant temperature, pressure and chemical potential of salt as the system is brought from an initial (i) to a final (f) configuration. The difference in free energy is

$$\Delta\mathcal{G} \ = \ -\int_{V^i}^{V^f} \Pi(V_{DNA})\ dV_{DNA}. \tag{3}$$

The excess or packing energy per unit length of the DNA helix can now be obtained as

$$\frac{\Delta\mathcal{G}}{L} = -\sqrt{3}\int_{D_i}^{D_f}\ \Pi(D)\ DdD, \tag{4}$$

where D is the interhelical spacing assuming the DNA array is at least locally hexagonal. Since the DNA osmotic pressure decays exponentially at small and intermediate values of D, a finite density interval is sufficient to evaluate the above integral to satisfactory accuracy. We have taken D_i corresponding to the concentration 15mg/ml (data not shown on Fig.1), which marks the onset of the condensed (anisotropic) DNA phase [23].

Since thermal fluctuations are contributing to the free energy it is reasonable to express the calculated free energy per unit length, $\frac{\Delta\mathcal{G}(D)}{L}$, in its "natural" units of kT per persistence length $\mathcal{L}_p$ (≈ 500 Å). In these units one can write

$$\frac{(\Delta\mathcal{G}(D)/kT)}{L/\mathcal{L}_p} = \frac{\mathcal{L}_p}{\zeta(D)}, \tag{5}$$

where $\zeta(D)$ is the contour length of DNA associated with kT of packing energy in the condensed phase.

2 RESULTS

2.1 *Osmotic Stress Measurements*

The dependence of osmotic pressure on the concentration of the unoriented DNA subphase has been investigated in detail [10, 11, 17]. The corresponding interhelical spacings were obtained by measuring the first order x-ray diffraction peak on unoriented DNA samples assuming local hexagonal packing symmetry. This assumption was verified experimentally in the high density region (I) (see Fig.1) through the existence of weak higher order reflections and now by observing well developed six-fold symmetric bond orientational order (see section 2.2).

Similar measurements were performed on oriented samples that show the same interaxial spacing (or density) dependence on Π as the unoriented samples (see Fig. 1) and thus have the same free energy, within experimental error. There are two distinct regions in the $\Pi - D$ curve. In the high pressure regime, the interhelical distance does not depend on the salt concentration . The forces between helices in this region were interpreted as resulting from water - mediated structural forces [10]. At lower pressures a sensitivity of D to salt concentration is clearly discernible. The effective decay length for the interhelical interactions, however, is about twice the predicted Debye screening length [20] for salt concentrations < 1.0 M, where electrostatic interactions are not overwhelmed by hydration forces. The two scaling regimes of the osmotic pressure are separated by a narrow crossover region in the $\Pi - D$ curve at about $32 - 34$ Å.

2.2 *Packing Symmetry*

The two regimes in the osmotic pressure curve are also clearly evident in the qualitative characteristics of the X-ray diffraction on oriented samples (see Fig. 2). For oriented samples of DNA in the high osmotic pressure regime (I) the cross section of the first order interaxial diffraction peak with the DNA helical axis oriented parallel to the incoming beam is a circular ring with six-fold modulation in the intensity which clearly reflects the six-fold symmetric long range bond orientational order of the underlying DNA lattice Fig.2 (inset). Azimuthal modulation of the first order diffraction peak at close DNA spacings has been observed previously in neutron diffraction studies [15] with fibers of NaDNA and LiDNA at low excess salt content. As the osmotic pressure is lowered the six-fold modulation of the first order diffraction peak disappears and is unobservable below the transition, 32-34 Å, region (see inset Fig.2) in the $\Pi - D$ curve. For spacings less than 35 Å the changes in the six-fold modulation of the diffraction peak were reversible. However, once the bond orientational order is lost, it cannot be regained by simply increasing the osmotic pressure. The subsequent chain entanglement due to the looser nature of the

packing in this low pressure phase apparently precludes the reestablishment of long range bond orientational order. The details of the first order diffraction peak are irretrievably lost leading to a circular powder pattern.

The details of the azimuthal profile of the diffraction pattern were independent of the X-ray beam size up to cross sectional areas on the order of $\sim$ mm^2. The bond orientational order thus appears to be of very long range indeed. The translational order, on the other hand, estimated crudely from the radial linewidth of the first order diffraction peak [22] and extremely weak higher order reflections (J. Rädler, personal communication), appears to be of a much shorter range, on the order of several lattice spacings.

To quantify this change in orientational bond order, we have measured the azimuthal intensity distribution of the first order diffraction peak and extracted the corresponding Fourier coefficients shown in Fig.2. Generally the Fourier spectra showed pronounced peaks for $\mathcal{C}_n$ with $n = 0$ and 6, with typically a small, but discernible additional contribution from $\mathcal{C}_2$, most probably reflecting a slight misorientation of the x-ray beam direction and the average director of the oriented DNA sample. The extracted $\mathcal{C}_6$ coefficients, that are also corrected for misalignment, shows a gradual loss of lateral bond orientational order as the DNA density passes from the high to low osmotic pressure regimes .

The nature of the low osmotic pressure phase can be further ascertained by polarized light microscopy which clearly reveals the existence of a "fingerprint" texture characteristic of a cholesteric phase [21]. Though the pitch of the cholesteric phase varies with density of the DNA phase in the vicinity of I $\longrightarrow$ II transition, we were unable to quantify this accurately because the orientational domain sizes were, in general, small. Due to the high molecular weight of the DNA, the samples could not be manipulated by an applied external orienting magnetic field to increase the domain size.

2.3 *Phosphate Backbone Dynamics*

An earlier analysis of the $\Pi - D$ curve suggested that there was a relatively sudden change in lattice fluctuations, inferred from changes in x-ray scattering peak widths, within the 32-34 Å transition region, [11]. This change in motion can now be seen very clearly in the ^{31}P NMR spectra. The insert in Fig. 3 shows two ^{31}P NMR spectra - one within the high pressure regime and one in the low pressure, cholesteric phase - that clearly demonstrate a symmetry change in the effective tensor of chemical shift. While any quantitative relation between the values of the effective tensor of chemical shift, the spectral first moment, and the details of the molecular motions is highly model dependent, it is clear that there is a qualitative difference in the DNA dynamics between the two pressure regimes. We have quantified this change by analyzing the first moments of the ^{31}P NMR spectra, shown in Fig.3. If there are no other processes contributing to resonance broadening and since osmotically equilibrated samples are monophasic, the observed increase of the first

moment as the interaxial spacing decreases is due to a decreased mobility of DNA helices.

The molecules are obviously immobilized to a substantial degree in the high pressure phase though the spectral first moment does decrease somewhat , see Fig.3, as the 32-34 Å transition region is approached. In the low pressure region, the phosphate mobility appears to be significantly greater. The difference in the spectral first moments between the two pressure regimes suggests a drastic increase of motional amplitudes for the cholesteric phase but the mobility appears not to change substantially with density within this phase.

Typical principal values of the chemical shift tensor extracted from spectra at high applied osmotic stress are $\sigma_{xx} \approx -60$ ppm, $\sigma_{yy} \approx -5$ ppm, and $\sigma_{zz} \approx 65$ ppm. Comparable values for essentially completely immobilized dry DNA, are -83 ppm, -22 ppm and 110 ppm (measured relative to 85% phosphoric acid as a standard) [16]. The effective tensor for DNA in the high pressure regime shows that phosphate motions are quite restricted. No fast rotation around one axis is present because this would have resulted in a tensor with axial symmetry.

3 DISCUSSION

3.1 *Structure and Dynamics*

This study, together with earlier measurements of intermolecular forces [10, 11], presents a departure from the usual gravimetric method of sample preparation. By bringing ordered phases into equilibrium with large "reservoirs" of salt-plus-polymer solutions rather than by making stoichiometric mixtures of salt, water and DNA, it is possible to set all the intensive thermodynamic variables associated with the resulting single liquid-crystalline phase.

These simultaneous measurements of the structure, motion and thermodynamic functions of DNA phases have focused on high density DNA phases (with interhelical spacings between about 25 and 55 Å) at one ionic strength (0.5 M NaCl). This density region extends from $\sim$ 120 mg/ml to $\sim$ 600 $\frac{mg}{ml}$. At lower densities there is a transition to a cholesteric phase from one of the (presumably) blue phases [24], while at higher densities there is a transition into a three dimensional crystal with a simultaneous B $\longrightarrow$ A transition in DNA conformation. For the long fragment DNA investigated here, the isotropic $\longrightarrow$ anisotropic transition is still quite remote ($\sim$ 10 mg/ml [23]).

The structural, dynamic, and osmotic stress data presented here are all consistent with the existence of two different DNA phases separated by a transition region at a DNA density of $\sim 320 - 360$ mg/ml, corresponding to interhelical spacings of $\sim 32 - 34$ Å. Previous work on short fragment (146 bp $\sim$ 500 Å long) DNA [4] also gave clear evidence for the existence of a series of structurally distinct regions as a function of DNA concentration. The transition from a cholesteric to a 2D-hexagonal phase for short fragment DNA was observed at $\sim$ 32 Å. Remarkably the $\sim$ 32 Å interaxial spacing is also close to the spacing from which Mn^{2+} or Co^{3+}-DNA collapses in a first order transition under osmotic stress [25]. Is this a distance at which the details of the chiral double-helical structure come to be sensed in molecular interaction?

What these experiments do not show clearly is the nature and the order of the transition between bond orientationally ordered and cholesteric phases. There is no detectable discontinuity (the accuracy of the measurement of the interhelical spacing in this regime of DNA densities is $\sim$ 1 Å) in the Π *vs.* interaxial separation curve that is seen when DNA makes a clear first-order transition [25]. This should not be taken as definitive evidence, however, that the transition is second order. An extremely narrow phase coexistence window could simply be a property of polymers in liquid crystalline mesophases [26]. The accuracy of the azimuthal scans of the first order diffraction peak as well as the first moment of the ^{31}P NMR spectra also precludes a definitive measure of the order of the transition.

3.2 *Free Energy and Intermolecular Forces*

The "osmotic stress" exerted by the excluded polymer is the rate of change of free energy with change in the amount of solution in the DNA phase, i.e., $\Pi = -\partial G/\partial V_{DNA}$. By integrating the osmotic pressure curve one thus obtains the change in the system free energy, Eq.4. In the insert to Fig. 1 we have plotted this free energy as a function of molecular separation. It is given in thermal units of kT per persistence length (see Eq.5), and spans a wide range of energy scales, from about kT per 2.5 Å at $\log(\Pi) \sim 8$ dynes/cm^2 to about kT per 100 Å at $\log(\Pi) \sim 6$ dynes/cm^2.

Previous work [17] has established that forces in the high pressure regime are dominated by exponentially decaying hydration interactions with a decay length $\lambda \sim 3 - 4$ Å that is basically independent of the ionic strength. In the low pressure regime, the interaxial spacing dependence on osmotic stress is also exponential, but the effective decay length is about twice the expected Debye decay length (at least for salt concentrations between 0.2 and ~ 0.8 M). The enhanced decay length and a rescaling of the strength of the interactions between DNA helices in this regime of DNA densities was shown to be due to the progressive onset of conformational disorder characterized by the fluctuations in the mean position of the molecules along the average director [17, 18] and deduced from the width of the interhelical x-ray scattering peaks. The switch between fluctuation enhanced forces and bare potentials was not found to be gradual, but rather quite abrupt as the DNA density passed the ~ 340 mg/ml limit [11], correlating nicely with emergence of longitudinal order between helices seen in the studies of Livolant *et al.* [4] on short fragment DNA, as well as with the onset of lateral bond orientational order and broadening of the phosphate NMR peak reported here.

The fluctuation-enhanced effective interactions observed in DNA arrays have the same origin as the effective interactions in smectic arrays. They are due to the interplay between conformational fluctuations and bare short range potentials [20]. The clearly emerging enhancement of electrostatic decay length to about twice the Debye length, not yet so easily seen in lipid bilayer smectic arrays, could be connected with the different dimensionalities of the two systems (2D periodicity *vs.* 1D periodicity).

3.3 *Perspectives and Directions*

Molecular interactions in DNA arrays, extracted from the measured osmotic pressure of the array, are expected [11] to vary with the interaxial spacing D as $\sim K_0(D/\lambda)$, where $K_0(x)$ is the modified Bessel function with asymptotic behavior $K_0(x) \approx (\pi/2x)^{1/2}\ e^{-x}$, with a decay length λ dependent on the salt concentration [17]. In this respect, as noted by Nelson [2], the interactions between helices in condensed DNA mesophases are formally and surprisingly closely related to the interactions between magnetic vortex lines in flux-

line lattices of high-T_c superconductors which, apart from the lack of hard core repulsions, share the same form of interaction potential.

The existence of a line (polymer) hexatic phase, intermediate between a line crystal and a line liquid, was hypothesized by Marchetti and Nelson [28] specifically for the case of magnetic flux-line lattices. It appears that the bond ordered DNA phase (region I) described above is perhaps this type of intermediate phase. The transition from a line hexatic phase in DNA into one of the possible less ordered phases is complicated by the presence of chiral coupling in the molecular interactions at lower densities, leading to the cholesteric, not a line liquid, phase. The occurrence of line hexatic between the cholesteric and the crystalline (A-form DNA) phases makes it difficult to compare directly with existing theoretical predictions. Its existence nevertheless introduces a new possible scenario into the melting sequence of ordered polyelectrolyte arrays.

To say that DNA provides an opportunity to learn about liquid-crystals is not to say that it has already given clear answers to basic questions. What is the nature of the transition from a phase with well developed six-fold symmetric bond orientational order to a a skewed, cholesteric phase when the molecules are allowed to move apart? Why does this change in symmetry couple with the molecular motions that cause extra interaxial separation [11]? What is the nature of molecular packing in the long polymer cholesteric phase compared to the more common twisted nematic phases of shorter molecules? These combined structural studies [4], osmotic stress measurements of free energies, and x-ray & NMR probes of molecular disorder and motion now provide a direction and an opportunity for further development of systematic theoretical analyses.

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Figure captions

Figure 1. Osmotic pressure of DNA, stressed by solutions of PEG (20,000 MW) at different concentrations, as a function of the interhelical spacing for 0.5 M NaCl DNA, for wet-spun, highly oriented DNA • and for condensed unoriented DNA ◇. No significant difference between the macroscopically oriented and unoriented samples is observed over the osmotic stress region investigated. The upper continuous curve represents a fit to the experimental data with bare hydration forces at high pressures (region I) and fluctuation enhanced screened Coulombic interactions in the low pressures region (II), while the lower curve represents a hypothetical osmotic pressure dependence if the underlying forces were a simple sum of bare hydration and screened Coulomb interactions, neglecting the contribution from DNA conformational disorder. The dotted vertical region represents the phase boundary between the bond orientationally ordered and the cholesteric phases. A DNA density scale in mg/ml is also given in order to facilitate comparison with previous work. The insert presents the free energy in units of kT per persistence length ($\sim$ 500 Å or $\sim$ 150 base pairs). $\frac{\mathcal{L}_p}{\zeta(D)} = 1$ corresponds to one persistence length per kT while 100 corresponds to one hundredth of a persistence length (or $\sim$ 1.5 base pairs) per kT of interaction energy.

Figure 2. The dependence of the bond orientational order parameter $\mathcal{C}_6$ on the interaxial spacing in the region of the high-pressure to low-pressure transition. The vanishing of the long range bond order is clearly visible. Six-fold modulation in the first order diffraction peak seen directly in the scattering patterns (inset, for left to right, $\log(\Pi) = 7.80, 7.51, 6.795$ dynes/cm^2), gradually weakens and disappears below the transition region (shown by the dotted area) identified in the $\Pi - D$ shown in Fig. 1. This loss of bond orientational order is reversible provided the interhelical spacing does not exceed the transition region ($\sim$ 35 Å).

Figure 3. The first moment M_1 of the ^{31}P NMR spectra as a function of the interhelical separation D measured on the same (unoriented) samples as with x-ray scattering. There is a qualitative change in the shape of the NMR spectra (see inset) as the system goes through the high-pressure to low-pressure transition region (the dotted area). The inset shows two NMR spectra: at $\Pi = 7.98$ dynes/cm^2 corresponding to interhelical spacing of 26 Å (broad spectrum) and at $\Pi = 6.119$ dynes/cm^2 corresponding to interhelical spacing of 46 Å (narrow spectrum) and ionic strength 0.5 M NaCl. Much more motional freedom of DNA phosphates is evident at low pressures than high. The change in the behavior of the spectral linewidth at the transition region is quite abrupt.